

New Insights, Treatments, and Management Strategies for ADPKD: Role of Vasopressin Antagonists

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Alterations in intracellular calcium homeostasis and cyclic adenosine 3',5'-phosphate likely underlie the increased cell proliferation and fluid secretion in polycystic kidney disease. Hormone receptors that affect cyclic adenosine 3',5'-phosphate and are preferentially expressed in affected tissues are logical treatment targets. There is a sound rationale for considering the arginine vasopressin V2 receptor as a target. The arginine vasopressin V2 receptor antagonists OPC-31260 and tolvaptan inhibit the development of polycystic kidney disease in cpk mice and in three animal orthologs to human autosomal recessive polycystic kidney disease (PCK rat), autosomal dominant polycystic kidney disease (Pkd2-/WS25 mice), and nephronophthisis (pcy mouse). PCK rats that are homozygous for an arginine vasopressin mutation and lack circulating vasopressin are markedly protected. Administration of V2 receptor agonist 1-deamino-8-D-arginine vasopressin to these animals completely recovers the cystic phenotype. Administration of 1-deamino-8-D-arginine vasopressin to PCK rats with normal arginine vasopressin aggravates the disease. Suppression of arginine vasopressin release by high water intake is protective. V2 receptor antagonists may have additional beneficial effects on hypertension and chronic kidney disease progression. A number of clinical studies in polycystic kidney disease have been performed or are currently active. The results of phase 2 and 2-3 studies indicate that tolvaptan seems to be safe and well tolerated in autosomal dominant polycystic kidney disease. A phase 3, placebo-controlled, double-blind study in 18- to 50-yr-old patients with autosomal dominant polycystic kidney disease and preserved renal function but relatively rapid progression, as indicated by a total kidney volume >750 ml, has been initiated.

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The polycystic kidney disease (PKD) proteins are multifunctional interacting proteins that are essential to maintain the differentiated phenotype of the tubular epithelium. Reduction in one or more of these proteins below a critical threshold results in a phenotypic switch characterized by altered protein trafficking and targeting, increased cell-matrix and reduced cell-cell adhesiveness, increased rates of proliferation and apoptosis, loss of planar polarity, and expression of a secretory phenotype. Increased cell proliferation and loss of planar polarity are responsible for cyst initiation, whereas increased fluid secretion and the persistent effects of factors that initiate cyst formation contribute to cyst growth (reviewed in reference [1]).

Molecular Mechanisms of Polycystic Kidney Disease

The proteins encoded by PKD1 (polycystin-1 [PC1]), PKD2 (polycystin-2 [PC2]), and PKHD1 (fibrocystin/polyductin [FC]) are membrane-associated proteins. PC2 is a transient receptor potential channel with high permeability to calcium (also known

as TRPP2). PC1 (also known as TRPP1) directly and FC indirectly interact with PC2 and modulate its channel activity (2-4). PC1 and FC have other functions that in turn may be modulated by PC2 and calcium. PC1, PC2, and FC are located in primary cilia (5-7). PC2 is also present in the endoplasmic reticulum, where it interacts with inositol triphosphate and ryanodine receptors (8-10). Together, these receptors are responsible for calcium release from intracellular stores. In primary cilia, the polycystin complex translates mechanical stimulation of the cilia into calcium entry, which triggers calcium-induced calcium release from the endoplasmic reticulum (Figure 1A). PC2 interacts with TRPV4, a likely component of the mechanosensory apparatus in primary cilia (11), and TRPC1, the strongest candidate component of the store-operated calcium channel (12). Reductions in the levels of PC or FC below a critical threshold disrupt intracellular calcium homeostasis (13-17). In renal tubular epithelial cells, intracellular calcium limits cAMP accumulation by inhibiting adenylyl cyclase 6 and possibly by activating phosphodiesterase 1 (18,19). In animal models of autosomal dominant (ADPKD) and recessive (ARPKD) PKD, the renal levels of cAMP are increased, likely as a result of the disruption of intracellular calcium homeostasis (20-24). cAMP stimulates chloride-driven fluid secretion (25) (Figure 1B). Whereas under normal conditions cAMP inhibits mitogen-activated protein kinase signaling and cell proliferation, in PKD or in conditions of calcium deprivation it stimulates cell proliferation in an src-, Ras-, and B-Raf-dependent manner (26-28). Cell proliferation

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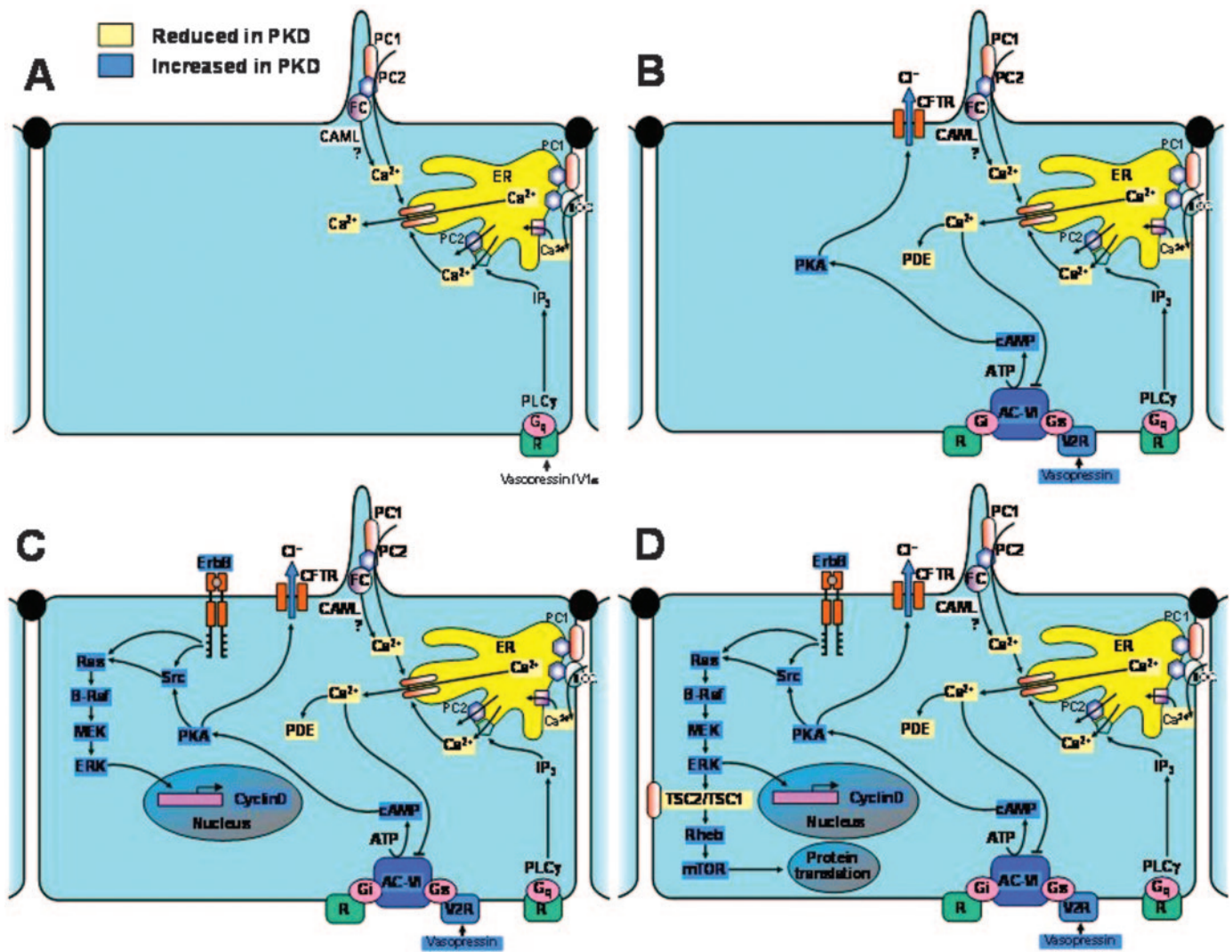


Figure 1. (A) Polycystin-1 (PC1), polycystin-2 (PC2), and fibrocystin/polyductin (FC) are located in primary cilia. PC2 is also present in the endoplasmic reticulum, where it interacts with inositol triphosphate (IP₃R) and ryanodine (RR) receptors. Together, these receptors are responsible for calcium release from intracellular stores. In primary cilia, the polycystin complex translates mechanical stimulation of the cilia into calcium entry, which triggers calcium-induced calcium release from the endoplasmic reticulum (ER). (B) Reductions in the levels of PC or FC below a critical threshold disrupt intracellular calcium homeostasis. Reduced calcium level at certain cellular domains enhances cAMP accumulation by increasing the activity of adenylyl cyclase 6 and possibly decreasing the activity of phosphodiesterase 1. cAMP stimulates chloride-driven fluid secretion. (C) Whereas under normal conditions cAMP inhibits mitogen-activated protein kinase signaling and cell proliferation, in PKD or in conditions of calcium deprivation it stimulates cell proliferation in an src-, Ras-, and B-raf-dependent manner. The proliferative effect of cAMP may be further enhanced by the stimulation of Erb-B receptors by EGF-like factors present in cyst fluid. (D) Mammalian target of rapamycin (mTOR) is also activated in cystic epithelium, likely as a result of a disrupted tuberlin-PC1 interaction or to extracellular signal-regulated kinase- or Akt-dependent phosphorylation of tuberlin that prevents its association with hamartin and inhibits its GTPase activating function for Rheb. AC-VI, adenylyl cyclase type VI; ATP, adenosine triphosphate; CAML, calcium modulating cyclophilin ligand; CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; G_i, G_i-type protein; G_q, G_q-type protein; IP₃, inositol triphosphate; MEK, mitogen-activated protein kinase kinase; PDE, phosphodiesterase; PKA, protein kinase A; PKD, polycystic kidney disease; PLC, phospholipase C; R, receptor.

may be further enhanced by stimulation of Erb-B by EGF-like factors that are present in cyst fluid (Figure 1C) (29). Mammalian target of rapamycin is also activated in cystic epithelium, likely as a result of a disrupted tuberlin-polycystin-1 interaction or to extracellular signal-regulated kinase- or Akt-dependent phosphorylation of tuberlin that prevents its association with hamartin and inhibits its GTPase activating function for Rheb (30) (Figure 1D).

Hormone Receptor-Targeted Interventions

The understanding of molecular mechanisms operative in PKD has identified a number of targets for therapeutic interventions that have been effective in animal models of the disease (31). A strategy to target hormone receptors that affect cAMP is attractive, given the restricted expression of particular

receptors in certain tissues and the central role of cAMP. There are a number of reasons for considering the use of arginine vasopressin (AVP) V2 receptor antagonists. The localization of the V2 receptors in the distal nephron and collecting duct (32) corresponds to the main site of cystogenesis in ARPKD and arguably in ADPKD (33). As a result of terrestrial adaptation, tetrapods are constantly subjected to the tonic action of vasopressin on V2 receptors. AVP is the main agonist of adenylyl cyclase in freshly dissociated collecting ducts (34). Patients with PKD have increased circulating levels of AVP (35–37). Animal models of PKD also have increased circulating levels of AVP as well as upregulation of AVP- and cAMP-dependent genes such as the V2 receptor and aquaporin-2 (20–22). In the past decade, several nonpeptide, orally bioavailable V2 receptors have been extensively investigated for the treatment of euvoletic and hypervolemic hyponatremia and found to be reasonably safe (38–42).

Preclinical Studies

In 1999, Gattone *et al.* (43) reported that the V2 receptor antagonist OPC-31260 had a marked protective effect on the development of PKD in the *cpk* mouse, a model of rapidly progressive cystic disease. To extend this observation, OPC-31260 was then used in three animal models orthologous to human ARPKD (PCK rat), ADPKD (*Pkd2*^{WS25/-} mouse), and adolescent nephronophthisis (*pcy* mouse) (20,21). Renal concentrations of cAMP are significantly increased in the three models, compared with wild-type animals. In PCK rats, the administration of OPC-31260 between 3 and 10 wk or between 10 and 18 wk of age significantly reduced the renal levels of cAMP, the activation of Ras and extracellular signal-regulated kinase, and the expression of the pro-proliferative isoform of B-Raf. This was accompanied by a marked inhibition of disease development, when administered between 3 and 10 wk of age, or of disease progression, when administered between 10 and 18 wk of age, as reflected by significant reductions in kidney volume, cyst and fibrosis volumes, plasma blood urea nitrogen (BUN), and mitotic and apoptotic indices. In *Pkd2*^{WS25/-} mice, the administration of OPC-31260 lowered the renal levels of cAMP, downregulated the expression of V2 receptor- and cAMP-dependent genes (V2 receptor and aquaporin 2), and markedly inhibited the development of PKD, as reflected by lower kidney weights, cyst and fibrosis volumes, plasma BUN levels, and mitotic and apoptotic indices. OPC-31260 was also protective in the *pcy* mouse (Figure 2). Because OPC-31260 is a weak antagonist for the human V2 receptor, a derivative with a higher affinity for the human V2 receptor (tolvaptan) has been under commercial development for the treatment of hyponatremia in humans (44). This antagonist was also effective in animal models of ARPKD, ADPKD, and nephronophthisis (22,45,46). Neither OPC-31260 nor tolvaptan had a beneficial effect on the development of fibropolycystic liver disease, which is consistent with the absence of V2 receptor expression in the liver.

To determine whether the protective effect of these drugs is indeed due to V2 receptor antagonism, we decided to generate PKD rats that lack circulating AVP and determine the effect of administering the exogenous V2 receptor agonist 1-deamino-8-

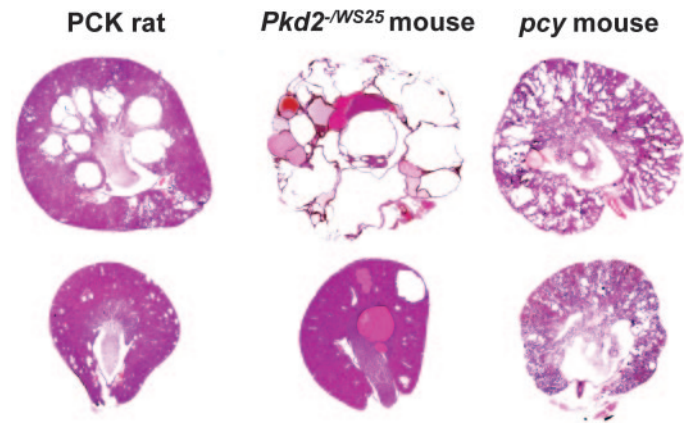


Figure 2. Kidney sections from PCK rats, *Pkd2*^{-/WS25} mice, and *pcy* mice untreated (top) or treated (bottom) with OPC-31260. Sections from PCK rats and *pcy* mice are representative kidney sections. Sections from *Pkd2*^{-/WS25} mice illustrate the most severe cystic disease in the control and treatment groups.

D-AVP (dDAVP) (47). For this purpose, we crossed Brattleboro rats, which are homozygous for a mutation in the *AVP* gene and lack circulating AVP, and PCK rats and intercrossed the F1 animals to create PCK rats with normal *AVP*, heterozygous or homozygous for an *AVP* mutation, as well as wild-type and Brattleboro controls. At 10 and 20 wk of age, PCK rats homozygous for an *AVP* mutation were almost completely protected, whereas *AVP* heterozygosity had no detectable protective effect. The administration of dDAVP between 12 and 20 wk of age completely recovered the cystic phenotype. The administration of dDAVP to PCK rats with normal *AVP* aggravated the development of the cystic disease (Figure 3).

Nagao *et al.* (48) offered PKD rats 5% sucrose in drinking water to increase water intake and suppress AVP levels. This strategy caused a marked amelioration of the renal cystic dis-

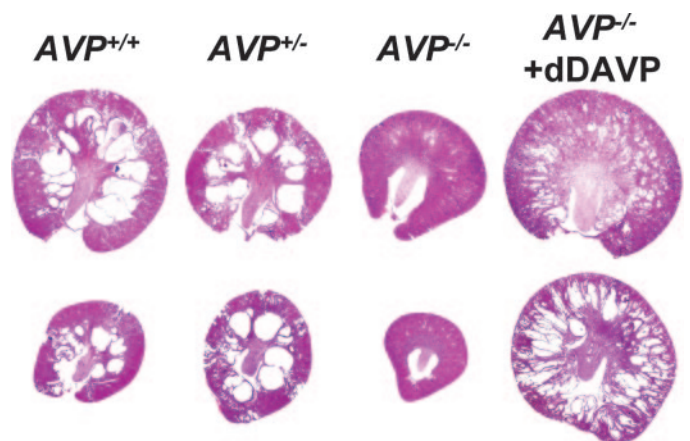


Figure 3. Representative kidney sections from 20-wk-old male (top) and female (bottom) PCK *AVP*^{+/+}, PCK *AVP*^{+/-}, and PCK *AVP*^{-/-} rats, and PCK *AVP*^{-/-} rats treated with 1-deamino-8-D-arginine vasopressin (dDAVP; 10 ng/h per 100 g body wt) using osmotic minipumps from 12 to 20 wk of age.

ease, supporting a central role for AVP and cAMP in cystic disease.

In addition to an effect on cystogenesis, V2 receptor antagonists may have beneficial effects on hypertension and chronic kidney disease (CKD) progression. In regard to hypertension, V2 receptor antagonists may have an antihypertensive effect by functionally downregulating amiloride-sensitive epithelial Na^+ channel, Na^+K^+ -ATPase, $\text{Na}^+\text{K}^+\text{2Cl}^-$ co-transporter, and thiazide-sensitive Na^+Cl^- co-transporter, thus reducing sodium reabsorption (49–53). Consistent with this is the observation that Brattleboro rats are resistant to the development of DOCA-salt hypertension and that chronic stimulation of vasopressin V2 receptors increases BP in the healthy rat and worsens hypertension in DOCA-salt-treated animals (54,55). Conversely, an argument could be made that V2 receptor antagonists could have a prohypertensive effect by inhibiting nitric oxide production in the collecting ducts and interfering with medullary vasodilation (56).

In regard to CKD progression, Bankir and colleagues (57,58) argued that V2 receptor activation, by increasing urea recycling from the collecting duct into the loop of Henle, reduces sodium concentration at the macula densa; inhibits tubuloglomerular feedback; stimulates renin release; and results in glomerular hyperfiltration, proteinuria, and renal damage. By interfering with this chain of events, V2 receptor antagonists might have a renoprotective effect similar to that of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers. In this regard, it is of interest that AVP deficiency and water loading inhibit CKD progression in five-sixths nephrectomized rats (59,60).

Clinical Studies

A number of clinical studies on the effect of tolvaptan in ADPKD have been completed or are currently active under the Tolvaptan Efficacy and Safety in Management of PKD and Outcomes (TEMPO) program. Phase 2a studies (248 and 249) have been completed, whereas two multicenter studies (250 and 251) and a single-center mechanistic study (260) are currently active.

In TEMPO 248, ascending single doses of tolvaptan (15, 30, 60, and 120 mg) were administered every 3 d and showed a dosage-dependent increase in urine output (61–63). Hypostenuria, a surrogate marker for vasopressin suppression, was sustained during 16 h after the administration of 30, 60, or 120 mg, but urine osmolality increased above 300 mOsm/kg in most patients 16 to 24 h after receiving 30 or 60 mg of tolvaptan. In TEMPO 249, different split doses of tolvaptan (15/15, 30/placebo, 30/15, 30/30 mg) were administered for 5 d at 8 a.m. and 4 p.m. to groups of patients (parallel-arm design) (61–63). Split-dose administration was more effective than a single dose in achieving sustained hypostenuria, and the mean urine output on the fifth day was lower than that on the first day of treatment, 4 to 6 compared with 6 to 7 L per 24 h.

Study 250 is an open-label study that consists of a titration phase and a fixed-dose phase (64). The initial dose of tolvaptan in the titration phase was 30/15 mg at 8 a.m. and 4 p.m., with the option to down-titrate to 15/15 if not tolerated. The dosage

was increased at weekly intervals to 45/15, 60/30, and 90/30 mg when tolerated. During the titration phase 96, 61, and 46% of the patients said that they could tolerate 45/15, 60/30, and 90/30 mg for the rest of their life. Urine osmolalities before the morning dose of tolvaptan were >300 mOsm/kg in 20 to 30% of patients who were taking 45/15, 60/30, or 90/30 mg/d, pointing to the difficulty in achieving sustained hypostenuria in all of the patients. On the basis of the results of the titration phase, participants were randomly assigned to high (60/30 mg) and low (45/15 mg) dosages of tolvaptan for the fixed phase that is planned to last 3 yr. At the time of a published interim report, at approximately half point into the trial, the mean premorning dose urine osmolality was <300 mOsm/kg. There was an initial, slight increase in serum creatinine that later declined toward baseline. Serum BUN significantly decreased, whereas there was a slight but significant increase in uric acid. Both systolic and diastolic BP tended to decrease with time, but this change is difficult to interpret in the absence of a control group. Five patients withdrew from the study, in four cases because of adverse events and one case because of noncompliance. One of the adverse events that led to discontinuation was deemed to be related to the drug, an increase in serum creatinine from 1.4 to 1.7 mg/dl that was rapidly reversible after discontinuation of the drug. The other adverse events that led to discontinuation included periorbital swelling, transient ischemic attack, and a benign pituitary microadenoma.

TEMPO 251 is a phase 3, placebo-controlled, double-blind study in 18- to 50-yr-old patients with preserved renal function but relatively rapid progression, as indicated by a total kidney volume >750 ml. The primary end point is renal volume change by magnetic resonance, and the secondary end point is time to multiple progression events. The duration of treatment is 3 yr. After a screening and a randomization visit, the masked medication is increased at weekly intervals from 45/15 to 90/30 mg/d if tolerated. After the titration phase, visits occur every 4 mo and magnetic resonance scans are obtained yearly.

Conclusions

Extensive animal studies suggest that AVP is a powerful modulator of cystogenesis, that inhibition of renal cAMP production accounts for the protective effect of V2 receptor antagonists, and that these drugs may afford additional benefits on hypertension and CKD progression. These studies provide a strong rationale for clinical trials using V2 receptor antagonists in ADPKD. Phase 2a studies have been completed. Two multicenter studies (an open-label study and a phase 3, placebo-controlled, double-blind study) are currently active.

Disclosures

None.

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